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Research paper

Manganese-enhanced MR imaging of brain activation evoked by noxious peripheral electrical stimulation

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HIGHLIGHTS

- Mn^{2+}-enhanced signals were observed in specific ROIs after peripheral stimulation.
- The ROIs were highly associated with noxious stimulation.
- MEMRI could delineate the functional areas of pain perception.

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ABSTRACT

As imaging technology develops, magnetic resonance imaging (MRI) has furthered our understanding of brain function by clarifying the anatomical structure and generating functional imaging data related to information processing in pain conditions. Recent studies have reported that manganese (Mn^{2+})-enhanced MRI (MEMRI) provides valuable information about the functions of the central nervous system. The aim of this study was to identify specific brain regions activated during noxious electric stimulation using high-resolution MEMRI. Male Sprague Dawley rats were divided into three groups: naïve, sham electrical stimulation, and noxious electric stimulation. Under urethane with α-chloralose mixture anesthesia, a catheter was placed in the external carotid artery to administrate 20% mannitol and manganese chloride (25 mM $MnCl_2$). Noxious electric stimulation (2 Hz, 10 V) was applied to the hind paw with a needle electrode. Stimulation-induced neuronal activation was detected using 4.7-T MRI. In response to noxious electrical stimulation, remarkable Mn^{2+}-enhanced signals were observed in the agranular insular cortex, auditory cortex, primary somatosensory cortex of the hind limb, and granular and dysgranular insular cortex, which correspond to sensory tactile electric stimulus to the hindpaws. These results indicate that the combination of MEMRI with activity-induced Mn^{2+}-dependent contrast can delineate functional areas in the rat brain.

1. Introduction

A critical evaluation of current, non-invasive brain-mapping techniques indicates that no one technique in isolation can adequately address the varied questions of interest to basic researchers and clinicians. For example, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) were used to reveal the sensory evoked responses or consequent mapping of sensory pathways in the brain [10,19,22]. However, evaluating brain activity related to pain using earlier MRI techniques has been quite difficult, as the activated and/or inactivated brain areas are small in a rat brain. Thus, mapping the whole-brain activity directly and,

Abbreviations: AI, agranular insular cortex; AuD, auditory cortex; GI+DI, granular and dysgranular insular cortex; S1HL, primary somatosensory cortex of the hind limb; S1ULp, somatosensory cortex of upper lip region; V1, visual cortex; DMPAG, dorsomedial periaqueductal gray; BBB, blood–brain barrier; CNS, central nervous system; MEMRI, manganese-enhanced MRI; Mn^{2+}, manganese; MRI, magnetic resonance imaging; ROI, region of interest.

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particularly, studying the reorganization of brain activity patterns over time in the same animal require further methodological developments.

In accordance with recent technical developments in this field, manganese (Mn^{2+})-based contrast agent provides a novel tool to investigate the neural circuits in vivo with a combination of neural networks and functions [4]. Basically, Mn^{2+} ions are analogs of calcium ions, so they can be anterogradely transported along the axon and cross synapses [16,21]. The degrees of Mn^{2+} ions accumulation and transportation in the CNS are mainly related to neural activity [20]. Mn^{2+} has paramagnetic ion characteristics and magnetic resonance systems can therefore be used to detect neuronal changes after different types of stimulation [17,18]. For example, Mn^{2+}-enhanced MRI (MEMRI) has been used to identify brains areas activated by noise exposure in mice, to differentiate between normal and ischemic heart regions in dogs [7], to image the rat visual cortex [6], and to detect alterations and study glaucoma in mouse models [2]. MEMRI has the potential to reveal structural alterations and neural activity patterns and provide functional information.

Recent neuroimaging studies of pain-related brain areas have shown similar functional reorganization in the anterior cingulate cortex (ACC), somatosensory cortex, thalamus, and insula [8,12,14]. These findings suggest that the brain undergoes widespread structural and functional changes following painful sensations, which may lead to the transition of acute to chronic and maladaptive pain signaling. However, the transition of pain signaling and the involved cortical and subcortical areas are not well understood. The present study was performed to use MEMRI to identify the specific Mn^{2+}-enhanced areas associated with hypersensitivity in rat brain after noxious peripheral electrical stimulation.

2. Materials and methods

2.1. Ethical statement

Male Sprague Dawley rats (250–300 g; DBL, Eumseong, Korea) were used. They were kept on a 12-h light/dark cycle (lights on at 7:00 a.m.) in a temperature (20–23 °C) and humidity (40–55%)-controlled environment. Rats were housed in groups of four with ad libitum access to food and water. A total of 30 rats were used for to assess $MnCl_2$-produced enhancement in MR signals after electrical stimulation. All animal experimental procedures were in accordance with the Guidelines for the Institutional Animal Care and Use Committee of Yonsei University Health System and were approved by the University ethical board.

2.2. Animal preparation

Three groups of rats (naïve, $n = 10$; sham electrical stimulation, $n = 10$; and noxious electric stimulation, $n = 10$) were used for this study. All rats were anesthetized with 3% isoflurane in a 1:1 oxygen-air mixture and injected with atropine (20 μg/kg) to avoid excessive mucus secretion in the trachea. Next a skin incision was performed on the right ventral aspect of the neck, and then the common carotid artery and external carotid artery (ECA) were gently exposed. The catheters for intracarotid infusion via ECA were cannulated using polyethylene tubing (PE-10) (Fig. 1). Rats were anesthetized with an injection of urethane (0.5 g/kg, intraperitoneal [i.p.]) and α-chloralose (25 mg/kg), and the isoflurane was discontinued. The depth of the anesthesia was determined by a lack of flexor response to hind-paw pinching, then the rats were mounted on a stereotaxic apparatus for MRI scanning. The heart and respiration rates were monitored during the experiments.

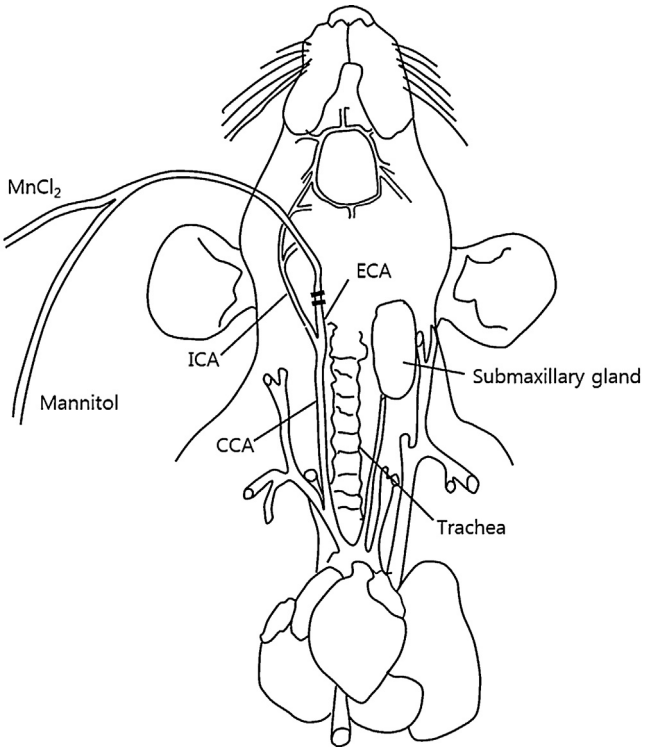

Fig. 1. Schematic depicting the experimental procedure for $MnCl_2$ and mannitol injections for MEMRI. $MnCl_2$ and mannitol were injected via a PE-10 catheter inserted into the external carotid artery (ECA). Injected drugs were delivered to the internal carotid artery (ICA) and ultimately diffused near the middle cerebral artery (MCA) in the circle of Willis.

2.3. Agent administration

Following catheter insertion, rats were secured inside the MR scanner, and drug administration and image acquisition were performed according to the activity-induced MEMRI paradigm [1]. To open the blood–brain barrier (BBB), 20% D-mannitol solution (5 ml/kg, Dai Han Pharm, Seoul, Korea) was injected via the right carotid artery after T1-weighted (T1W) image acquisition. Manganese chloride ($MnCl_2$–$4H_2O$, Sigma, St. Louis, MO, USA) dissolved to 25 mM in isotonic saline was infused via the right carotid artery at 500 μl/min using a syringe pump (SP120PZ, WPI, Sarasota, FL, USA) for the entire recording time. The $MnCl_2$ solution injection was started at the same time as MRI acquisition and was stopped after obtaining the final set of images. For sham electrical stimulation rats, an electrode was inserted in the left hind paw, but electrical stimulation was not delivered. For the electric stimulation group, noxious stimulations (10 V amplitude, 10 ms delay, 2 ms width, and 2 Hz) were applied to the left hind paw after $MnCl_2$ injection (Fig. 2).

2.4. MR imaging

MR imaging was performed using a 4.7-T horizontal spectrometer (Bruker BioSpin, Ettlingen, Germany) with a shielded gradient coil (65 mm diameter) and a transmit/receive surface coil (25 mm diameter). After tuning and shimming, T2 multislice spin echo sequence images were acquired for positioning. T1W images were taken at the same positions. T1W gradient-echo coronal images were obtained using the following parameters: repetition time = 200 ms, echo time = 10 ms, matrix size = 256 × 256, field of view = 40 × 30 mm^2, slice thickness = 1 mm, number of averages = 10, number of slices = 12, and interslice gap = 1 mm. The total acquisition time of each T1W image scan was 40 s. Twelve images were acquired for each set in the data acquisition paradigm,